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1) Estimates of variation and heritability for nodule mass and recovery of *Rhizobium japonicum* strain 110

Introduction: The increase of biological dinitrogen fixation has been an illusive and rather stochastic area of research for many soybean workers. One aspect of the symbiosis that merits more attention is the identification of genetic variability in host plants for the traits associated with fixation and the utilization of this variability in plant breeding programs. The purpose of the present study was to provide information on genetic variability for nodule mass and recovery of strain 110 in three soybean populations.

Materials and methods: The parents used to generate the three populations used in this study were plant introductions identified by Kvien et al. (1981) as showing diversity in their ability to form nodules with the indigenous *R. japonicum* strains (primarily serogroup 123). A rating system was set up on a scale of 1-6, 1 = low nodule mass to 6 = high nodule mass (mass, meaning visual estimates of nodule number and nodule size). PI 372415B and PI 68622 were given a rating of 6. PI 91119 and PI 189922 were classified as 1 and 2, respectively, for their amount of nodule mass. The cross PI 372415B x PI 91119 was made and 20 F₃-derived F₅ and F₆ lines from it constitute population 1. Population 2 was made up of 46 F₃-derived F₅ and F₆ lines from the cross PI 372415B x PI 189922. Population 3 contained 31 F₃-derived F₅ and F₆ lines from the cross PI 372415B x PI 68622. The 97 lines, the four parents and seven adapted varieties were planted in 1982 at Rosemount, St. Paul and Becker, MN. The experimental design consisted of four replications of a split-plot strip design. A peat-base inoculant containing *R. japonicum* strain 110 (an effective N₂-fixer) was applied with the seed at planting in one row of the two-row plot. Root cores were taken from each row in late July and ratings given according to the aforementioned scale. Sixteen nodules were taken at random from the sampled root systems and serotyped by the quick-test agglutination method (Damirgi et al., 1967) to identify the strain(s) of rhizobium forming each nodule.

Results and discussion: Previous researchers (Lawson, 1980; Gupta et al., 1982) subdivided a line's nodulation response into several components (i.e., nodules/plant and dry or fresh nodule weight/plant). Estimates of variances and heritabilities for these components differed between the two studies. In an attempt to further explore the potential sources of variability involved, three dissimilar populations and three diverse locations were used in the present study.

Estimates of means, ranges, variances and heritabilities for nodule mass for the three populations and three locations are presented in Table 1. Nodule mass/plant was significantly different among populations at Becker and St. Paul. The three locations were significantly different for nodule mass/plant. The means across populations were 1.89, 1.99 and 2.90 for St. Paul, Rosemount and Becker, respectively. Population 1 consistently had the highest nodule mass rating across all locations. The range in ratings

was largest at Becker. The heritability estimates on an entry-mean basis were consistent for each population across locations except in one instance. Estimates of heritability across locations based on entry-means were 40.73%, 67.94%, 57.67% for population 1, population 2 and population 3, respectively. Progress from selection for nodule mass should be possible if selection is based on replicated plots across locations. Use of the visual rating system for nodule mass as previously described in this paper showed a good correlation to the ranking of genotypes based on actual measurements of nodules/plant and fresh nodule weight. The use of this visual rating system would allow a much larger sample to be evaluated for nodulation ability and allow this evaluation to be done in the field. The inclusion of visual nodule mass as a selection criterion hinges on the relationship of nodule mass to yield. Some yield responses were noted in this study but the data are still being analyzed.

Previous research on the recovery of introduced rhizobium from soils containing large naturalized populations also show some divergence in conclusions. Kvien et al. (1981), in screening 85 diverse genotypes, found some genotypes that preferentially formed a significant percentage of their nodules with strain 110; however, a large amount of variability was present for recovery of strain 110 due to environmental effects. Kvien reported recoveries of inoculant strains as high as 40.0-50.0%. Lawson (1980) found location means for recovery of strain 110 in the range of 8.0% to 21.0%. Lawson reported a heritability estimate of 30.5% for the recovery of strain 110 across three locations. Ellis (1982) found inoculation with high rates of *Rhizobium japonicum* did not significantly increase the recovery of strain 110 in soybean nodules.

Table 2 contains estimates of means, ranges, variances and heritabilities for the recovery of strain 110. The three locations were significantly different for percent 110 recovered. Means averaged over the three populations were: 9.04%, 9.14% and 11.48% for Becker, Rosemount and St. Paul, respectively. Population 2 had the highest recoveries of strain 110 at all three locations. The widest range for recovery was 0.00% to 31.00%, which occurred at Becker. Large environmental variances were observed for recovery of strain 110 for all populations and all locations. The existence of a population-by-location interaction precluded presenting heritabilities over locations. Yield data are still being analyzed, but the narrow range of percent 110 recovered would limit the amount of yield response expected.

The interrelationship of the two traits included in this study has been examined in the past. Kvien et al. (1981) concluded that a line's recovery of strain 110 under field conditions was independent of the line's nodulation ability with the native rhizobia. In the present study, population 1 had the highest nodule mass/plant at all three locations, but showed the lowest recoveries of strain 110 across all three locations. Population 2 rated the poorest for nodule mass at Rosemount and St. Paul, but showed the highest recoveries of strain 110 at these two locations. However, correlations between nodule mass and the recovery of strain 110 were not statistically significant at any of the three locations.

Conclusions: Use of the visual rating system for improving nodule mass and the high heritabilities associated with its use offer hope for increasing the nodulation ability of lines currently being developed in breeding programs. The large environmental variances associated with the recovery of

Table 1. Estimates of mean, range, variances (phenotypic, genotypic, environmental) and heritability for nodule mass of uninoculated rows of soybeans

Character	Location	Population	Mean \pm S.E.	Range	Variances			Heritability
					Phenotypic	Genotypic	Environ.	
Nodule mass per plant*	Becker	1	3.30 \pm .12	2.0-6.0	1.100	.129	.974	34.63
		2	2.77 \pm .07	1.0-5.0	.961	.218	.743	53.99
		3	2.76 \pm .08	1.0-6.0	.720	.157	.563	52.75
	Rosemount	1	2.15 \pm .10	1.0-4.0	.803	.121	.682	41.51
		2	1.89 \pm .06	1.0-4.0	.710	.229	.481	65.55
		3	1.94 \pm .08	1.0-4.0	.699	.134	.565	48.62
	St. Paul	1	2.09 \pm .11	1.0-5.0	.914	-.022	.935	\approx 0.00
		2	1.60 \pm .06	1.0-4.0	.533	.165	.368	64.20
		3	2.03 \pm .08	1.0-4.0	.746	.067	.679	28.12

*(1 = low nodule mass, 6 = high nodule mass).

Table 2. Estimates of mean, range, variances (phenotypic, genotypic, environmental) and heritability for recovery of strain 110 of *Rhizobium japonicum*

Character	Location	Population	Mean \pm S.E.	Range	Variances			Heritability
					Phenotypic	Genotypic	Environ.	
Recovery of strain 110*	Becker	1	7.47 \pm .68	0.0-31.3	36.69	-2.09	38.78	\approx 0.00
		2	9.02 \pm .48	0.0-31.3	41.81	-0.26	42.07	\approx 0.00
		3	9.92 \pm .61	0.0-31.3	46.66	6.66	40.00	39.96
	Rosemount	1	7.64 \pm .87	0.0-26.7	27.47	-4.66	32.13	\approx 0.00
		2	9.34 \pm .54	0.0-21.4	24.23	2.42	21.81	30.73
		3	8.95 \pm .58	0.0-25.0	19.27	0.55	18.72	10.50
	St. Paul	1	10.23 \pm .87	0.0-20.0	24.74	6.09	18.65	56.65
		2	11.31 \pm .73	0.0-31.3	47.14	0.88	46.25	7.13
		3	11.21 \pm .75	0.0-25.0	37.16	-1.49	38.65	\approx 0.00

* % of nodules containing strain 110

strain 110 and the low recoveries observed may limit the progress in selection for this trait. The choice of location in which to screen genotypes for genetic variability for traits associated with nitrogen fixation is an important determinant of the amount of improvement that can be realized.

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2) Effect of seed production environment on genetic differences in cold tolerance during germination.

Genetic variability for cold tolerance in soybeans has been reported by a number of researchers (Hillsman et al., 1977; Holmberg, 1973; Hume and Jackson, 1981; Hwang, 1979; Lambert, 1978, Littlejohns and Tanner, 1976; Spehar, 1977). Selection experiments for cold tolerance generally have pursued either vigorous germination and emergence or success in flowering and pod set. Neither is fully understood, although they appear to be independent of each other (Holmberg, 1973; Hume, personal communication). Examination of results of previous studies reveals that rank order changes among studies are not uncommon. This report summarizes some of our work in attempting to assess possible environmental effects on the prediction of genetic differences in germination cold tolerance.

We took 20 genotypes and grew them in five locations in Minnesota (St. Paul, Rosemount, Waseca, Lamberton and Morris) in 1981 and 1982. The genotypes used are listed in Table 1. Most of the named varieties have been grown in Minnesota at some time during the last 30 years, except 'Fiskeby V', a Swedish variety selected for superior cold tolerance during flowering and pod set, and 'Salut 216', a Russian variety reported by some to be cold tolerant. II-62-173 is a Minnesota line reported to have cold tolerance during emergence (Lambert, 1978). The PI lines were chosen based on their cold tolerance ratings by Spehar (1977), and based on differences in percent protein, percent oil or seedweight after USDA data. Spehar reported

Table 1. Soybean genotypes tested, their maturity groups and mean values obtained for germination index (with standard deviation and ranks), percent protein, percent oil, and hundred seedweight in grams

Genotype	M.G.	G.I.	Std. dev.	Rank	Prot.	Oil	Sdwt.
Altona	00	11.67	3.13	17	43.12	18.22	15.67
Blackhawk	I	8.13	2.09	3	41.66	18.19	15.08
Clay	0	9.56	3.32	11	41.91	19.13	14.88
Evans	0	11.51	2.66	18	41.66	18.38	14.23
Fiskeby V	000	12.70	3.24	20	42.21	18.58	14.28
Grande	0	12.12	2.97	19	40.04	18.13	20.84
Grant	0	8.97	2.72	6	42.04	17.60	15.48
Hodgson 78	I	10.51	3.15	12	41.89	18.66	15.82
McCall	00	8.72	2.28	5	40.12	18.84	13.25
Salut 216	00	9.44	2.47	9	42.31	18.30	13.21
Swift	0	9.28	2.72	7	40.37	18.23	15.28
II-62-173	0	9.28	2.62	8	41.60	18.57	13.13
PI 89001	0	10.88	3.06	13	43.24	17.49	19.40
PI 153320	0	9.57	2.76	10	42.96	18.38	14.90
PI 180507	00	7.32	2.91	1	44.58	18.94	14.77
PI 229330	0	11.83	3.63	16	47.71	13.52	10.33
PI 248401	0	10.91	2.91	14	44.09	16.84	13.70
PI 257432	0	8.41	2.49	4	42.85	17.41	13.48
PI 257433	0	7.57	2.02	2	42.45	18.24	14.01
PI 258384	0	11.09	3.43	15	43.12	17.50	15.06

that PI 89001 and PI 258384 were slow cold germinators, and PI 229330, PI 248401, PI 257432 and PI 257433 were fast cold germinators. PI 180507 was rated as only slightly faster than average, but was a significant outlier from the regression of protein on oil.

Fifteen live seeds from seed samples collected in each environment were put in petri dishes containing 60 g sand and 30 ml water within a growth chamber held at 10°C. A germination index (hereafter G.I.) was calculated based on the number of days for the first 10 seeds to have the radicle break through the testa:

$$G.I. = \frac{\sum (\text{no. seeds newly germ'd})(\text{no. days to that reading})}{(\text{total no. seeds germ'd with that reading})}$$

Three replications were run. Percent protein, percent oil and hundred-seed weight were also obtained from the seed samples.

Analyses of variance for seed from each environment (location and year) showed that significant differences among genotypes existed for the G.I. from every environment except one (Waseca in 1982) which had experienced a severe drought. Combined analyses of variance revealed highly significant differences both years for genotypes and the genotype x location effect. Locations were significant in 1982 but not in 1981. Year had a nonsignificant effect.

Large rank order changes in the genotype G.I. values were common among seed from different environments. Correlations among means of genotypes for each environment are shown in Table 2. Seventeen of the 45 possible correlations were significant, although many of these had small r^2 values. We also regressed the mean values for each of the 1982 environments on the mean values for the 1981 environments. Of the 25 possible regressions, only two were significant. This ambiguity across environments is also reflected in the very large standard deviation values for the G.I. means based on seed from all ten environments (Table 1). Looking at Table 1, it can also be noted that PI 180507, PI 229330, PI 248401 and Salut 216 all performed differently than reported in other studies.

Table 2. Correlation coefficients among soybean genotypes from different environments

	RO81	WA81	LA81	MO81	STP82	RO82	WA82	LA82	MO82
STP81	.62**	.17	.48*	.15	.34	.23	.10	.27	.19
RO81		.32	.40	.19	.22	.25	.49*	.23	.49*
WA81			.27	.56**	.22	.09	.36	.39	.49*
LA81				.38	.41	.54*	.36	.64**	.47*
MO81					.18	.44*	.46*	.73**	.60**
STP82						.46*	.42	.20	.25
RO82							.65**	.48*	.31
WA82								.43	.51*
LA82									.62**

*,** Denote significance at .05 and .01 levels of probability, respectively.

STP = St. Paul; RO = Rosemount; WA = Waseca; LA = Lamberton; MO = Morris.

Our regressions between G.I. values and percent protein and hundred-seed weight were nonsignificant, and the regression between G.I. and percent oil was significant ($r^2 = 0.017$, however). Hwang (1979) reported similar results. It is interesting that the fastest germinator on a mean basis was also the outlier for high protein with high oil.

In line with others' reports, there is no apparent relationship between maturity group and germination cold tolerance. The poor performance of Fiskeby V tends to buttress the hypothesis that summer and spring cold tolerance are independent.

When the mean genotype values over all 1981 locations were used to predict the mean values over all 1982 locations, the regression was highly significant ($r^2 = 0.56$). In another study, not discussed further here, we compared percent emergence, an Emergence Index (E.I.), height and dry weight under early spring planting conditions with G.I. values, and found no significant correlations for G.I. values from any one seed source environment, but some significant correlations (for the E.I.) when G.I. values were the means over all seed source environments. Thus, we conclude that germination cold tolerance needs to be evaluated with seed from multiple seed production environments to be of reliable predictive value for any genetic differences in cold tolerance.

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